Elemental Micro-Imaging of Human Brain Tumors Using SR-XRF and XANES Techniques

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The recent development of synchrotron radiation based microprobe beamlines has enabled spatially resolved XRF (X-ray fluorescence) and XANES (X-ray absorption near edge structure spectroscopy) at cellular and subcellular level. Synchrotron radiation XRF (SR-XRF) microprobe analysis as a multielemental analytical method was applied to simultaneous imaging of chemical elements in human brain tumors. Micro-XANES technique which provides information primarily about geometry and oxidation state was used to determine the chemical state of Fe in brain gliomas.

The SR-XRF and XANES measurements were performed at the bending magnet beamline L at HASYLAB. In case of SR-XRF the primary photon energy was set to 17 keV. To reduce the beam size the high flux capillary was used. The beam was focused to a size of 15 μm in diameter. Two-dimensional maps of selected element distribution were determined. The step sizes applied for mapping were equal to 15 μm both horizontally and vertically. The time of acquisition was equal to 10 s per pixel. The measurements were carried out in air. The characteristic X-ray lines were measured by the Vortex SDD detector from SII Nano Technology USA Inc. The measurements of either NIST standard reference materials (SRM 1833 and SRM 1832) or thin film XRF calibration standards were performed for the determination of masses per unit area of elements.

In the XANES measurements the Si-111 monochromator was applied. The measurements were carried out in the air. For the selected points the full XANES profiles were collected. In this case the absorption spectra near Fe K-edge were measured for the energy range from 7.05 to 7.50 keV. The energy step increments were equal to: 5; 1; 0.3 and 10 eV for the following energy ranges (respectively): 7.05¸7.08 keV; 7.08¸7.105 keV; 7.105¸7.14 keV and 7.14¸7.50 keV. The measurement time was 10 s for all analyzed energy points. Besides the tissue samples, the micro-XANES spectra were measured also for reference materials (thin sections of natural minerals pyrite and andradite, bought at Micro-Analysis Consultants Ltd. as wel as powder form of $FeSO_4*7H_2O$, $Fe_2(SO_4)_3*nH_2O$). The characteristic X-ray lines of Fe were measured with the use of HPGe detector. Additionally oxidation states imaging involved scanning a sample in two dimensions with spatial resolution equal to 15 μm on each direction. The monochromator was set to the appropriate energy for the edge of Fe oxidation states. Mapping of the different forms of iron was performed at energies of 7.127 keV (Fe^{2+}), 7.136 keV (Fe^{3+}) and 7.500 keV (total Fe).

The research allowed detection of P, S, Cl, K, Ca, Fe, Cu, Zn, Br and Rb in human brain tumors.

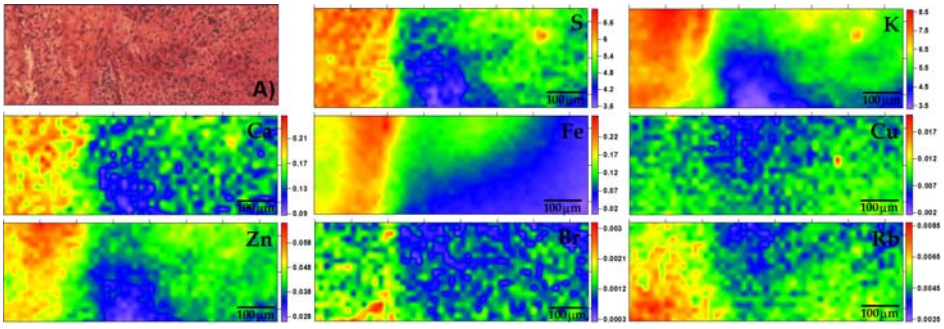

Figure 1: Distribution of selected elements in glioblastoma multiforme in comparison with microscopic view of hematoxylin-eosin stained tissue section (A).

The topographic analysis enabled to determine two-dimensional distribution of elements in characteristic tissue structures in case of cancerous tissues. The selected results were shown in Fig. 1.

The comparison of Fe XANES spectra measured in cancerous tissue and reference materials were presented in Fig. 2a. All the XANES spectra obtained for the tissue samples are situated between the spectra measured for reference materials containing Fe in the second and third oxidation state. For more precise determination the energies of the pre-edge peak maximum and the first inflection point of the main edge were calculated. It allowed to found that most of the Fe XANES spectra of glioma tissue are located closer to the spectrum obtained for $Fe_2(SO_4)_3$*nH_2O. This indicates that trivalent ferric Fe is more abundant form of iron in the points examined in brain glioma samples. However, taking into account inhomogeneity of the tissue structure the knowledge based on the results from random points seems to be insufficient. Therefore, the micro-imaging of iron oxidation states were performed. The mapping of different chemical form of iron allowed to found areas were bivalent or trivalent iron compounds were dominant. The maps of distribution (see Fig. 2b) of iron oxidation states were not correlated with location of cancer cells.

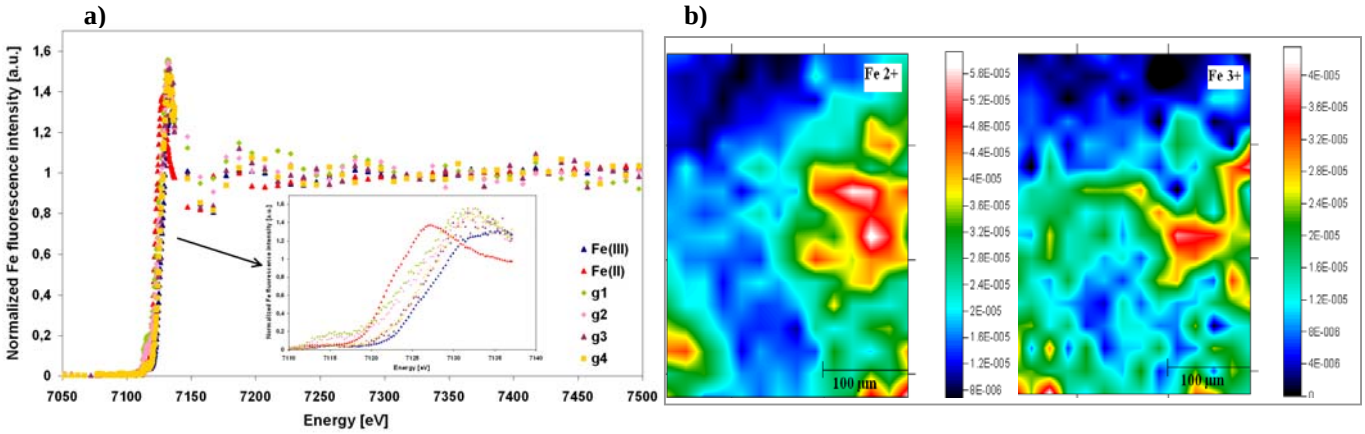

Figure 2: Fe XANES analysis of brain glioma tissue. a) The Fe XANES spectra acquired in selected points of different cases of glioma tissues (g1-g4) and reference materials. b) Scanning X-ray mocroprobe determination of iron oxidation state distribution in brain glioma tissue. Data presented in arbitrary units.

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